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RESEARCH ARTICLE

PRELIMINARY PHYTOCHEMICAL AND ANTHELMINTIC ACTIVITY OF *Phellinus wahlbergii*

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Abstract:

The aim of present study was the evaluation of preliminary phytochemical and anthelmintic activity of *Phellinus wahlbergii* extract from different solvents in experimental adult earthworm's *Pheritima posthuma*. The preliminary phytochemical properties of *Phellinus wahlbergii* fruiting bodies will be providing useful information in regard to the presence of active secondary metabolites such as alkaloids, flavonoids, glycosides, phenolics, steroids and triterpenoids and methanol extract at 60mg/ml show potent anthelmintic activity, paralysis time within 31.12 min and death time 52.24 min. The standard drug (Albenazole) shows paralysis within 21.98 min and time of death 59.92 min in all the three solvents extracts.

Key Words: *Phellinus wahlbergii*, Albendazole, *Pheritima posthuma*, Anthelmintic activity and Helminths.

Introduction

For millennia, mushrooms have been valued as edible and medical provisions for humankind. Medicinal mushrooms (MM) have an established history of use in traditional ancient therapies. Contemporary research has validated and documented much of the ancient knowledge. The interdisciplinary field of science that studies MMs has been developed and increasingly demonstrates potent and unique properties of compounds extracted from a range of mushroom species in the last three decades. Modern clinical practice in Japan, China, Korea, Russia and several other countries rely on mushroom-derived preparations (Mizuno, 1999; Chang, 1999; Wasser and Weis, 1999; Reshetnikov *et al.*, 2001; Van Griensven, 2009; Wasser, 2010a).

The benefits of mushroom compounds on different clinical conditions have attracted the interest of the scientific community in the last decade in order to understand the molecular mechanisms responsible for their actions. Several classes of mushroom compounds such as proteins, polysaccharides, lipopolysaccharides and glucoproteins have been classified as molecules that have potent effects on the immune system. They may restore and augment immunological responses of host immune effector cells, but they have no direct cytotoxic effect on tumors (Chihara *et al.*, 1969, 1970).

Helminths infections are among the most common infection in human beings and a major degenerative disease in world today, affected at least 1/3 people world population. The Helminths parasites are mainly live in human body intestinal tract, but they are also live in tissue, or their larvae migrate in to the tissue (Tripath, 2003). Anthelmintics are drug that either kill (vermicides) or, reduce the number of helminthes parasites in the intestinal tract or tissues of the body [Sharma, 2007].

The medical symbol which is used worldwide denotes even today to the modern world, about the success of the first treatable parasitic infection which is known to the human race (*Dracunculus medinensis*) (Poinar, 1983). Helminthiasis is mostly seen in the children of the tropical inhabitants of a low socioeconomic status. The available drugs like Albendazole, though they are capable of a broad spectrum action against the intraluminal parasites and the tissue parasites, have limitations for use in pregnancy and in children who are younger than 2 years of age (Katzung and Bertram, 2009). Because of the high prevalence rate of helminthiasis since ages, traditional and folklore medicines for it have been in use.

The present study was therefore conducted to evaluate the anthelmintic activity of different solvent extracts of *Phellinus wahlbergii*. Earthworms were used to test the anthelmintic activities since they bear anatomical and

physiological resemblance to the intestinal roundworm parasites of human beings (Thorn *et al.*, 1977 and Vigar, 1984).

Material and Methods

The methodology which was adopted to evaluate the anthelmintic activities of the mushroom *Phellinus wahlbergii* is as follows:

Mushrooms material

The *Phellinus wahlbergii* were collected from evergreen forest region which is located in Kigga village, Sringeri (T), Chickmagalur (D), Karnataka, during the month of August 2012 to September 2012. The *P. wahlbergii* of mushroom was picked from the litter and decaying wood surface, with help of forceps and then they were cleaned and air dried in an oven at 40°C for 48 h, dried mushroom samples were powdered mechanically for further use. Identification was done by comparing their morphological, anatomical and physiological characteristics according to (Kirk *et al.*, 2008). The voucher specimen has been deposited at the herbarium of Mycology Laboratory, Department of Applied Botany, Kuvempu University, Jnana Sahyadri, Shankaraghatta-577451, Shimoga district, Karnataka, India.

Chemicals

All the solvents viz., petroleum ether, chloroform and methanol used in this study were purchased from Hi-Media Laboratories Pvt. Ltd, of analytical grade.

Extract preparation

The dried *Phellinus wahlbergii* was pulverized into coarse powder in a grinder machine. The 200gm of dried mushroom powder extracted with petroleum ether, chloroform and methanol solvents by Soxhlet apparatus. Solvents from each sample was filtered, squeezed off and evaporated off under reduce pressure in a rotary evaporator to obtain crude extract. All the extracts are suspended in 1% Acacia in normal saline used as a vehicle for present study.

Worm collection and authentication

Indian adult earthworms (*Pheritima posthuma*) were used to study anthelmintic activity. The earthworms were procured from the Department of Horticulture Shimoga, Karnataka were identified in the Department of Applied Zoology, Kuvempu University, Jnana Sahyadri, Shankaraghatta-577451, Shimoga district of Karnataka.

Standard drug

For present study Albendazole had taken as standard drug. The concentration of standard drug was prepared in 1% gum Acacia in normal saline to give 10mg/ml concentration.

Preliminary phytochemical screening

Qualitative phytochemical analysis of *Phellinus wahlbergii* extracts (petroleum ether, chloroform and methanol) were carried out using standard methods (Tiwari *et al.*, 2011). The extracts obtained in Soxhlet extractor were dissolved with their respective solvents.

Anthelmintic investigation

The earthworms were used to determine anthelmintic activity of mushroom powder extracts, the earthworm divided into five groups, each group consisting six earthworms. The earthworms were first treated with 1% gum Acacia in normal saline, than treated with Albendazole (10mg/ml), petroleum ether, chloroform and methanol extract (20, 40 and 60mg/ml) extract of mushroom powder. Observations are made for the time taken to paralysis and death of individual worms. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worm lost their motility followed with fading away of their body color (Turner, 1965).

Results and Discussion

Phytochemical investigation

The qualitative phytochemical screening of *Phellinus wahlbergii* has revealed the presence of various secondary metabolites of therapeutic importance namely alkaloids, flavonoids, glycosides, phenolics, steroids and

triterpenoids whereas absent of lignins, tannins and saponins (Table 1). The result shows that all extracts contain the highest secondary metabolites. Several of these constituents may possibly be responsible for the mushrooms antimicrobial activity. Flavonoids, for instance, have been of interest to the scientific community because of recent reports on their antiviral, antifungal, anti-inflammatory, and cytotoxic (Aguinaldo *et al.*, 2004) and have also shown that antibacterial, anti-HIV property (Evans *et al.*, 2002).

Anthelmintic activity

In earthworms, the methanol and chloroform extracts of mushroom powder (*Phellinus wahlbergii*) show significant anthelmintic activity. The anthelmintic effect of mushroom powder extract is show (Table-2).

The extract shows dose dependent activity. The methanol extracts (60mg/ml) show paralysis within 31.12 min and time of death 52.24 min while the chloroform extracts (60mg/ml) show paralysis within 50.44 min and time of death 93.45 min. Similarly the methanol extracts (40mg/ml) show paralysis within 42.34 min and time of death 86.88 min. While chloroform extracts (40mg/ml) show paralysis within 56.34 min and time of death 99.47 min. lastly at 20mg/ml paralysis time 59.92 and 69.3 min respectively and death time 98.12 and 103.67 in both methanol and chloroform extracts. Same time petroleum ether extracts at 20, 40 and 60mg/ml shows moderate paralysis 72.27, 68.63 and 52.11 min respectively and death time 102.36, 97.89 and 89.76 respectively.

The standard drug (Albenazole) shows paralysis within 21.98 min and time of death 59.92 min in all the three solvents extracts. The observation of result show that the anthelmintic activity of methanol extract is more potent compare to the chloroform and petroleum ether extract. The literature reports reveal that caffeine, glycosides, alkaloids, thiobromine, polysaccharides, gallotannic acid, vitamins (C, E and K), steroids, amino acids, phenolics, flavanoides and triterpenoids. Present in the mushroom extracts know to possess anthelmintic activity. In the present investigation also the observed anthelmintic potential of test extract may be due to presence of similar phytoconstitutes, which was evident by preliminary process.

Table 1: Preliminary phytochemical analysis of secondary metabolites of *Phellinus wahlbergii*

Tests	Extracts			
	Petroleum ether	Chloroform	Methanol	Aqueous
Test for Alkaloids				
Mayer's test	+	+	+	+
Wagner's test	-	-	-	-
Dragendroff's test	-	-	-	-
Test for Flavonoids				
Flavonoid test	+	+	+	+
Zinc-hydrochloric acid reduction test	-	-	-	-
Ferric chloride test	-	-	-	+
Alkaline reagent test	-	-	-	-
Lead acetate test	-	-	+	-
Pew's Shinoda and NaOH test or Shinoda's test	-	-	-	-
Test for Glycosides				
KellarKiliani test	+	+	+	+
Molisch's test	+	+	+	+
Raymond's test	-	-	-	-
Bromine water test	-	-	-	-
Legal's test	-	-	-	-
Test for Lignins				
Furfuraldehyde test	-	-	-	-
Test for Phenolics				
Phenol test	+	+	+	+
Ellagic acid test	-	-	-	-
Hot water test	-	-	-	-
Test for Steroids				
Salkowski's test	+	+	+	+
Test for Tannins				
Sodium chloride test	-	-	-	-

Gelatin test	-	-	-	-
Ferric chloride test	-	-	-	-
Test for Triterpenoids				
Salkowski's test	-	+	-	-
Test for Saponins				
Foam test	-	-	-	-

Note: '+' = Present, '-' = Absent

Table 2: Anthelmintic activity of *Phellinus wahlbergii*

Treatment	Petroleum ether		Chloroform		Methanol	
	TTP in minutes (Mean and SD)	TTD in minutes (Mean and SD)	TTP in minutes (Mean and SD)	TTD in minutes (Mean and SD)	TTP in minutes (Mean and SD)	TTD in minutes (Mean and SD)
Control	-	-	-	-	-	-
Standard (10 mg/ml)	21.98	59.92	21.98	59.92	21.98	59.92
20	72.27	102.36	69.3	103.67	59.92	98.12
40	68.63	97.89	56.34	99.47	42.34	86.88
60	52.11	89.76	50.44	93.45	31.12	52.24

Note: 'TTP' = Time taken for Paralysis, 'TTD' = Time taken for Death.

CONCLUSION

From this study, we can say that the preliminary phytochemical and anthelmintic properties of *Phellinus wahlbergii* fruiting bodies will be providing useful information in regard to the presence of active secondary metabolites and methanol extract of mushroom powder have beneficial anthelmintic effect. Further pharmacological and biochemical investigation will clearly elucidate the mechanism of action and will be helpful in projecting this mushroom powder as a therapeutic target in helminthes research.

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